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A²
Infect. Immun., 61:3578-3582 (1993)), a smooth lipopolysaccharide (LPS) layer (Cross *et al.*, In: Bacteria, Complement and the Phagocytic Cell, Vol. H24, F. C. Cabello and C. Pruzzo, eds., Springer-Verlag, Berlin, pp. 319-334 (1988)), and certain OMPs including TraT (Montenegro *et al.*, J. Gen. Microbiol. 131:1511-1521 (1985); Moll *et al.*, Infect. Immun. 28:359-367 (1980)), Iss (Binns *et al.*, Infect. Immun., 35:654-659 (1982); Chuba *et al.*, Mol. Gen. Genet., 216:287-292 (1989)), and OmpA (Weiser *et al.*, Infect. Immun. 59:2252-2258 (1991)). The absence of capsule as a complement-resistance mechanism in disease-associated avian *E. coli* isolates suggests that such isolates must employ other means to avoid the killing effects of complement.

Please replace the paragraph at page 17, line 17 to page 18, line 2, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, with notations to indicate the changes made.

A^H
It is known to the art that while most of the serotypes or *E. coli* isolated from poultry are pathogenic only for birds, a few are also associated with disease conditions in other animals (Gross, Colibacillosis, In: Diseases of Poultry, Hofstad *et al.*, (eds.), The Iowa State University Press, pp. 270-278 (1984)). For instance, serotype O78:K80 is commonly isolated from cattle and sheep. Moreover, serotypes of *E. coli* frequently isolated from septicemic poultry are not among those pathogenic for humans. Since *E. coli* causing infections in birds can also be found in other animals, the term "avian *E. coli* iss nucleic acid sequence," as used herein, refers to nucleic acid sequences that are present in *E. coli* that may be present and pathogenic in birds as well as other animals. Nucleic acid sequences encoding avian *E. coli* Iss polypeptides can therefore be derived from and/or detected in nonavian as well as avian sources. For example, a nucleic acid sequence encoding avian *E. coli* Iss polypeptide can be derived from or detected

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Q4 in a farm animal known or believed to be naturally or experimentally infected by a virulent, septicemia-causing avian *E. coli*, including, for instance, poultry, cattle, or mink.

Please replace the paragraph at page 18, lines 5-19, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, with notations to indicate the changes made.

Q5 A nucleic acid molecule encoding an Iss polypeptide can be identified and isolated using standard methods, as described by Sambrook *et al.*, Molecular Cloning; A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989). For example, polymerase chain reaction can be employed to isolate and clone *iss* genes. "Polymerase chain reaction" or "PCR" refers to a procedure or technique wherein amounts of a preselected piece of nucleic acid, RNA and/or DNA, are amplified as described in U.S. Patent No. 4,683,195. Generally, sequence information from the ends of the region of interest or beyond is employed to design oligonucleotide primers. These primers will be identical or similar in sequence to opposite or complimentary strands of the template to be amplified. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and cDNA transcribed from total cellular RNA, bacteriophage or plasmid sequences and the like, to yield an amplification product. See also, Mullis *et al.*, Cold Harbor Symp. Quant. Biol., 51:263 (1986); Erlich, ed., PCR Technology (Stockton Press, NY, 1989).

Please replace the paragraph at page 19, lines 24 to page 20, line 17, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, with notations to indicate the changes made.

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Alternatively, DNA libraries may be probed using the procedure of Grunstein and Hogness Proc. Natl. Acad. Sci. USA, 72:3961 (1975), or other available techniques as described in Sambrook *et al.*, Molecular Cloning; A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989). Briefly, in this procedure, the DNA to be probed is immobilized on a membrane (*e.g.*, nitrocellulose or nylon filters) denatured, and prehybridized with a buffer containing 0-50% formamide, 0.75 M NaCl, 75 mM Na citrate, 0.02 % (wt/v) each of bovine serum albumin, polyvinyl pyrrolidone, and Ficoll, 50 mM Na phosphate (pH 6.5), 0.1% SDS, and 100 micrograms/ml carrier denatured DNA. The percentage of formamide in the buffer, as well as the time and temperature conditions of the prehybridization and subsequent hybridization steps depends on the stringency required. Oligomeric probes which require lower stringency conditions are generally used with low percentages of formamide, lower temperatures, and/or longer hybridization times. Both non-radioactive and radioactive techniques can be utilized. Probes containing more than 30 or 40 nucleotides such as those derived from genomic sequences generally employ higher temperatures, *e.g.*, about 40° - 42°C, and a high percentage, *e.g.*, 50%, formamide. Following prehybridization, 5'-³²P-labeled oligonucleotide probe is added to the buffer, and the filters are incubated in this mixture under hybridization conditions. After washing, the treated filters are subjected to autoradiography or a non-radioactive technique such as DIGOXIGENIN D/UTP labeling kit (Boehringer Mannheim, Indianapolis, Ind.), to show the location of the hybridized probe; DNA in corresponding locations on the original agar plates is used as the source of the desired DNA.

Please replace the paragraph at page 26, line 25 to page 27, line 8, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, with notations to indicate the changes made.

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Q1

The transformation procedure used depends upon the host to be transformed. Bacterial transformation by direct uptake generally employs treatment with calcium or rubidium chloride (Cohen Proc. Natl. Acad. Sci. USA, 69:2110 (1972)). Yeast transformation by direct uptake may be carried out using the method of Hinnen *et al.* Proc. Natl. Acad. Sci., 75:1929 (1978). Mammalian transformations by direct uptake may be conducted using the calcium phosphate precipitation method of Graham and Van der Eb (Virology, 52:456 (1973)), or the various known modifications thereof. Other methods for the introduction of recombinant polynucleotides into cells, particularly into mammalian cells, that are known in the art include dextran-mediated transfection, calcium phosphate mediated transfection, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the polynucleotides into nuclei.

Please replace the paragraph at page 30, lines 1-11, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, with notations to indicate the changes made.

Q2

Commonly used prokaryotic control sequences include the Beta-lactamase (penicillinase) and lactose promoter systems, the tryptophan (trp) promoter system (Goeddel *et al.* Nucleic Acids Res., 8:4057 (1980)) and the lambda-derived P_L promoter and N gene ribosome binding site (Shimatake *et al.* Nature, 292:128 (1981)) and the hybrid tac promoter (De Boer *et al.* Proc. Natl. Acad. Sci. USA, 80(3):21-5 (1983)) derived from sequences of the trp and lac UV5 promoters. The foregoing systems are particularly compatible with *E. coli*. If desired, other prokaryotic hosts such as strains of *Bacillus* or *Pseudomonas* may be used with appropriate control sequences. Although the promoters cited above are commonly used, other microbial promoters known in the art, are also suitable.

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Please replace the paragraph at page 31, lines 4-13, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, with notations to indicate the changes made.

Q^a Control sequences for yeast vectors are known in the art and include promoters for the synthesis of glycolytic enzymes (Hess *et al.* Adv. Enzyme Reg, 7:149 (1969)); (Holland *et al.* Biochemistry, 17:4900 (1978)), including the promoter for 3 phosphoglycerate kinase (Hitzeman *et al.* J. Biol. Chem., 255:12073 (1980)). Terminators may also be included, such as those derived from the enolase gene (Holland *et al.* J. Biol. Chem., 256:1385 (1981)). Particularly useful control systems are those that comprise the glyceraldehyde-3 phosphate dehydrogenase (GAPDH) promoter or alcohol dehydrogenase (ADH) regulatable promoter, terminators also derived from GAPDH, and if secretion is desired, leader sequence from yeast alpha factor.

Please replace the paragraph at page 31, line 22 to page 32, line 5, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, with notations to indicate the changes made.

Q¹⁰ Mammalian cell lines available as hosts for expression are known in the art. Suitable host cells for expressing Iss in higher eukaryotes include the following: monkey kidney CVI line transformed by SV40 (COS-7, ATCC CRL 1651); baby hamster kidney cells (BHK, ATCC CRL 1651); Chinese hamster ovary-cells-DHFR (described by Urlaub and Chasin, PNAS, 77:4216 (1980, USA)); mouse sertoli cells (TM4. Mather, J. P., Biol. Reprod., 23:243-252 (1980)); monkey kidney cells (CVI ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2 HB 8065); mouse mammary tumor (MMT

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Q10 060652, ATCC CCL 51); rat hepatoma cells (HTC. M1. 54. Baumann *et al.*, J. Cell Biol., 85:1-8 (1980) and TRI cells (Mather *et al.*, Annals N.Y. Acad. Sci., 383:44-68 (1982)).

Please replace the paragraph at page 33, lines 1-17, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, with notations to indicate the changes made.

Q11 Other systems for expression of eukaryotic or viral genomes include insect cells and vectors suitable for use in these cells. These systems are known in the art, and include, for example, insect expression transfer vectors derived from the baculovirus *Autographa californica* nuclear polyhedrosis virus (AcNPV), which is a helper-independent, viral expression vector. Expression vectors derived from this system usually use the strong viral polyhedrin gene promoter to drive expression of heterologous genes. Currently the most commonly used transfer vector for introducing foreign genes into AcNPV is pAc373. The vector pAc373 also contains the polyhedrin polyadenylation signal and the ampicillin-resistance (amp) gene and origin of replication for selection and propagation in *E. coli*. Many other vectors, known to those of skill in the art, have also been designed for improved expression. These include, for example, pVL985 (which alters the polyhedrin start codon from ATG to ATT, and which introduces a BamHI cloning site 32 base pairs downstream from the ATT; *See* Luckow and Summers Virology, 170:31 (1989)). Good expression of nonfused foreign proteins usually requires foreign genes that ideally have a short leader sequence containing suitable translation initiation signals preceding an ATG start signal.

Please replace the paragraph at page 33, lines 18-26, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, with notations to indicate the changes made.

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Q12 Methods for the introduction of heterologous DNA into the desired site in the baculovirus virus are known in the art. (See Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555; Smith *et al.* Mol. & Cell Biol., 3:2156-2165 (1983); and Luckow and Summers, *supra* (1989)). For example, the insertion can be into a gene such as the polyhedrin gene, by homologous recombination. Insertion can also be into a restriction enzyme site engineered into the desired baculovirus gene. The inserted sequences may be those which encode all or varying segments of the polyprotein, or other open reading frames ("ORFs") which encode viral polypeptides.

Please replace the paragraph at page 34, line 28 to line 35-4, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, with notations to indicate the changes made.

Q13 The Merrifield method is an established and widely used method. It is described in the following references: Stewart *et al.*, Solid Phase Peptide Synthesis, W. H. Freeman Co., San Francisco (1969); Merrifield, J. Am. Chem. Soc., 85:2149 (1963); Meienhofer in Hormonal Proteins and Peptides, Vol. 2, C. H. Li, ed., (Academic Press, 1973), pp. 48-267; and Barany and Merrifield in "The Peptides," Vol. 2, E. Gross and F. Meienhofer, eds., Academic Press (1980), pp. 3-285.

Please replace the paragraph at page 35, lines 5-26, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, with notations to indicate the changes made.

Q14 The Merrifield synthesis method commences from the carboxy-terminal end of the peptide using an alpha-amino protected amino acid. Fluorenylmethyloxy-carbonyl (Fmoc) or t-butyloxycarbonyl (Boc) protective groups can be used for all amino groups even though other protective groups are

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Q14 suitable, and the first protected amino acids can be esterified to chloromethylated polystyrene resin supports. The polystyrene resin support is preferably a copolymer of styrene with about 0.5 to 2% divinyl benzene as a cross-linking agent which causes the polystyrene polymer to be insoluble in certain organic solvents. See Carpino *et al.*, J. Org. Chem., 37:3404 (1972); Chang, Int. J. Peat. Pro. Res., 11:246 (1978); and Merrifield, J. Am. Chem. Soc., 85:2149 (1963). The immobilized peptide is then N-protected and other amino acids having protected amino groups are added in a stepwise manner to the immobilized peptide. At the end of the procedure, the final peptide is cleaved from the resin, and any remaining protecting groups are removed by treatment under acidic conditions, for example, with a mixture of hydrobromic acid and trifluoroacetic acid. Alternatively, the cleavage from the resin may be effected under basic conditions, for example, with triethylamine, where the protecting groups are then removed under acidic conditions. The cleaved peptide is isolated and purified by means well known in the art, for example, by lyophilization followed by either exclusion or partition chromatography on polysaccharide gel media such as Sephadex G-25, or countercurrent distribution. The composition of the final polypeptide may be confirmed by amino acid analysis after degradation of the polypeptide by standard means.

Please replace the paragraph at page 58, lines 24-30, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, with notations to indicate the changes made.

Q15 A *cvaC* probe was prepared by digesting plasmid pHK11 (Dr. R.E. Wooley, University of Georgia, Athens, GA) with the restriction enzymes *EcoRI* and *BglII*, which were obtained from Promega Corp. (Madison, WI.) to yield a 1.9 kb fragment (Gilson *et al.*, J. Bacteriol. 169:2466-2470 (1987)). To obtain a *traT* probe (Moll *et al.*, Infect. Immun., 28:359-367 (1980); Montenegro *et al.*, J.

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Q15
Gen. Microbiol., 131:1511-1521 (1985)), the plasmid pKT107 (Dr. F.C. Cabello, New York Medical College, Valhalla, NY) was digested with *Bst*EII to yield a 700 bp fragment.

Please replace the paragraph at page 63, lines 17-23, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, with notations to indicate the changes made.

Q16
Isolates were stab-inoculated into LB agar and incubated overnight at 37° C. Table 3 indicates positive and negative control organisms. Colonies were transferred to charge-modified nylon membranes (QIABRANE Nylon Plus membrane, QIAGEN, Inc., Chatsworth, Calif.) by the method of Grunstein and Hogness (Grunstein *et al.*, Proc. Natl. Acad. Sci. (USA), 72:3961-3065 (1975)). The colonies were lysed and the DNA denatured. Membranes were then stored and sealed in plastic bags (GibcoBRL, Gaithersburg, MD) at 4° C.
